

^1H , ^{13}C , and ^{15}N resonance assignments of subunit F of the A_1A_0 ATP synthase from *Methanosarcina mazei* Gö1

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Abstract Energy coupling between the A_1 ATPase of archaea type A_1A_0 ATP synthase and its integral membrane sub-complex A_0 occurs via the stalk part, formed by the subunits C, D and F. To provide a molecular basis of the energy coupling, we performed NMR studies. Here, we report the assignment of the subunit F.

Keywords A_1A_0 ATP synthase · A_1 ATPase · *Methanosarcina mazei* Gö1 · Heteronuclear NMR

Biological context

A key component in cellular bioenergetics is the ATP synthase. The enzyme from archaea represents a new class of ATPases, the A_1A_0 ATP synthases. They are composed of two domains, a membrane-embedded electrically-driven motor, A_0 , and a chemically-driven motor, A_1 . Adenosine triphosphate (ATP) is synthesized or hydrolyzed on the A_1 headpiece, consisting of an A_3B_3 domain. Previously, the 2.55 Å and 1.5 Å crystal structure of these major nucleotide-binding subunits A (Maegawa et al. 2006) and B (Schäfer et al. 2006a), respectively, have been solved, which provide important insight into nucleotide-binding positions of the catalytic A_3B_3 headpiece of this complex. The energy provided for or released during that process inside the A_3B_3 sector is transmitted to the membrane-bound A_0 domain. The energy coupling between the two active

domains occurs via the so-called stalk part, an assembly composed by the subunits C, D and F (Müller and Grüber 2003). Most recently, the low-resolution shape of subunit F of the A₁A₀ ATP synthase from the archaeon *Methanosarcina mazei* G₀1 in solution was determined by small angle X-ray scattering, showing that subunit F is an elongated molecule in solution with two distinct domains (Schäfer et al. 2006b). The subunit-subunit interaction of F inside the A₁A₀ ATP synthase in the presence of chemical crosslinker was studied as a function of nucleotide binding, demonstrating movements of F relative to the nucleotide-binding subunit B (Schäfer et al. 2006b) and supporting its function as an essential coupling element inside the enzyme during ATP hydrolysis and synthesis. However, the lack of any high resolution structural data for the stalk elements makes it particularly difficult to establish the molecular mechanism for the coupling of ATP hydrolysis to ion translocation. Thus, to understand the function of subunit F of the *M. mazei* G₀1 A₁A₀ ATP synthase in more detail two- and three-dimensional heteronuclear (¹³C, ¹⁵N) NMR spectroscopy has been performed. Here we present ¹H, ¹³C, and ¹⁵N resonance assignments of this protein.

Methods and experiments

Protein preparation

The cloning of the pET9d-His6 vector containing the DNA fragment, which encodes subunit F plus six His-residues at the N-terminus, has been described recently (Schäfer et al. 2006b). The uniformly ¹⁵N- and ¹⁵N/¹³C-labeled His₆- subunit F samples were expressed in *E. coli* BL21 (DE3) cells using M9 minimal media containing ¹⁵NH₄Cl, or ¹⁵NH₄Cl plus [U-¹³C]-glucose. The protein was purified by Ni²⁺-NTA affinity- and ion-exchange chromatography (Resource Q (6 ml), Amersham Biosciences) as described (Schäfer et al. 2006b). For NMR studies, the protein samples in 25 mM sodium phosphate pH 6.5 have been concentrated to 0.5–1 mM using Centriprep YM-10 (3 kDa molecular mass (MM) cut off) spin concentrators (Millipore).

NMR spectroscopy

All NMR spectra were recorded at 288 K on Bruker AV600 spectrometer equipped with a cryoprobe accessory. Backbone ¹H, ¹⁵N and ¹³C resonance were assigned using data from 2D ¹H-¹⁵N HSQC, 3D HNCA, HNCACB, CBCACONH, HNCOCA and HNCO spectra (Fig. 1). The

side chain ^1H and ^{13}C were obtained from 3D HCC(CO)NH-TOCSY, 3D (H)CC(CO)NH-TOCSY, HCCH-TOCSY, (H)CCH-TOCSY, 3D ^{15}N - ^1H -NOESYHSQC (Sattler 1999; Simon and Sattler 2004). All spectra were processed with NMRPipe (Delaglio et al. 1995) and analyzed by SPARKY (Goddard and Kneller 2003).

Assignments and data deposition

All non proline ^{15}NH correlations have been assigned, except for Phe²⁴, Gly⁹², Ser⁹³, Glu⁹⁸ and Asp¹⁰⁷ which were absent in the 2D $^1\text{H}/^{15}\text{N}$ -HSQC spectra recorded.

Assignment ambiguities were resolved using NOE data derived from the ^{15}N -NOESY-HSQC. No resonance assignments were made for the N-terminal hexahistidine-tag residues His³-His⁸ and for the residues Met¹ and Lys² due to their flexibility. In total more than 95% of backbone, 91% of side chain hydrogen and 80% of side chain carbon resonance assignments were made. The predicted fold using CSI (Wishart and Sykes 1994) consists of five α -helices, four β -sheets and NOE data from the ^{15}N -NOESY-HSQC are consistent with this. The absence of Phe²⁴ amide resonance could be due to the presence of weak intensity of ^{15}NH correlation of neighboring residue Gly²³, suggestive of enhanced amide exchange at this position. However, NOE data suggest this region is in fact α -helical. The initial calculated structure of subunit F confirms and supports the missing assignments, as this protein exhibits a rigid N-terminal domain comprising first 90 amino acid residues and a detached flexible C-terminal extension containing 20 amino acid residues. Almost all the unassigned resonances were lying in the C-terminal flexible domain. The presence of Gly and Ser rich in the C-terminal would favor rapid amide exchange rate in the C-terminal regions of F subunit. For the reasons mentioned above no resonance assignments could be made for Gly⁹² and following are the missing backbone assignments for the residues— $^{13}\text{C}'$ - Ser⁹¹, Ser⁹³, Thr⁹⁴, Glu⁹⁸, Glu¹⁰² and Ala¹⁰³ and H^α - Arg⁹⁷. The ^1H , ^{13}C and ^{15}N resonance assignments have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 15046.

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Fig. 1 2D ^1H - ^{15}N -HSQC spectrum of subunit F of the A_1A_0 ATP synthase from *M. mazei* Göl. The spectrum was recorded at 288 K on a Bruker Avance 600 MHz spectrometer. The assignments for resolved backbone residues are labeled with one letter amino acid code and residue number. The peaks marked by an asterisk (*) indicate the folded side-chain $\text{N}^\epsilon\text{H}$ protons of Arg

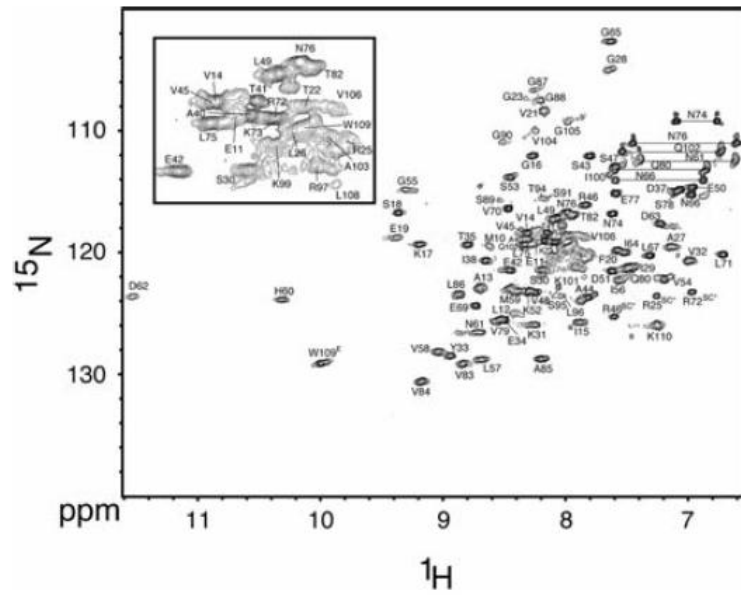


Fig.1